Biotechnological Applications of Hemicellulosic Derived Sugars: State-of-the-Art

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Abstract Hemicellulose is the second most abundant polysaccharide in nature, after cellulose. As a substrate, it is readily available for the production of value-added products with industrial significance, such as ethanol, xylitol, and 2, 3-butanediol. Hemicellulose is a heterogeneous carbohydrate polymer with a xylose-linked backbone connecting to glucose, galactose, mannose, and sugar acids. In general, it represents about 35% of lignocellulosic biomass. It is estimated that the annual production of plant biomass in nature, of which over 90% is lignocellulose, amounts to about 200×10^9 tons per year, where about $8–20 \times 10^9$ tons of the primary biomass remains potentially accessible. Hemicellulose, which is generally 20–35% of lignocellulose amounts to nearly ~70×10^9 tons per year. Continuous efforts by researchers in the last two decades have led the way for the successful conversion of hemicellulose into fermentable constituents by developed candidate pretreatment technologies and engineered hemicellulase enzymes. A major challenge is the isolation of microbes with the ability to ferment a broad range of sugars and withstand fermentative inhibitors that are usually present in hemicellulosic sugar syrup. This chapter aims to explore and review the potential sources of hemicellulose and their degradation into fermentable sugars, as well as advocating their conversion into value-added products like ethanol, xylitol, and 2, 3-butanediol.

Keywords Hemicellulose · Ethanol · Xylitol · 2, 3-Butanediol · Hydrolysis · Fermentation

1 Introduction

Biomass in the form of cellulose, hemicellulose, and lignin provides a means of collecting and storing solar energy, and hence represents an important energy and material resource [1–3]. After cellulose, hemicellulose is the principal fraction of the

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plant cell wall that could serve as a potential substrate for the production of value-added products under optimized conditions [4]. In general, the secondary cell walls of plants contain cellulose (40–80%), hemicellulose (10–40%), and lignin (5–25%). The arrangement of these components allows cellulose microfibrils to be embedded in lignin, much as steel rods are embedded in concrete to form reinforced concrete [5]. The composition of hemicellulosic fractions from different natural sources is summarized in Table 1.

The carbohydrate fraction of the plant cell wall can be converted into fermentable monomeric sugars through acidic and enzymatic (hemicellulase/cellulase) reactions, which have been exploited to produce ethanol, xylitol, and 2, 3-butanediol via microbial fermentation processes [1, 4, 12]. In the hemicellulosic fraction of the plant cell wall, xylan is the major backbone, linking compounds like arabinose, glucose, mannose, and other sugars through an acetyl chain [4]. They can be characterized as galactomannans, arabinoglucuronoxylans, or glucomannans based on their linkage with the main xylan backbone [13].

Thermal, chemical, and enzyme-mediated processes and combinations thereof are being explored in order to obtain monomeric components of hemicellulose with maximum yield and purity. The depolymerization of hemicellulose by chemical or enzyme-mediated processes yields xylose as the major fraction and arabinose, mannose, galactose, and glucose in smaller fractions [12]. This sugar syrup can be converted into ethanol; xylitol; 2, 3-butanediol (2, 3-BD); and other compounds [4]. The use of hemicellulose sugar as a primary substrate for the production of multiple compounds of industrial significance is summarized in Fig. 1.

A wide variety of microorganisms are required for the production of metabolites from hemicellulosic-derived sugar syrup. The ability to ferment pentoses is not widespread among microorganisms and the process is not yet well-established in

Table 1 Cell wall composition among various lignocellulosic sources considered for biofuel (% of dry material)

	Cellulose	Hemicellulose*					
Lignocellulosic source	Glucan	Xylan	Arabinan	Mannan	Galactan	Lignin	References
Sugarcane bagasse	40.2	22.5	2.0	0.5	1.4	25.2	[6]
Wheat straw	32.1	19.5	2.8	0.6	1.1	20	[7]
Corn stover	37.5	21.7	2.7	0.6	1.6	18.9	[8]
Switch grass	34.2	22.8	3.1	0.3	1.4	19.1	[7]
Pine wood	44.8	6.0	2.0	11.4	1.4	29.5	[9]
Aspen wood	48.6	17.0	0.5	2.1	2.0	21.4	[9]
Spruce wood	41.9	6.1	1.2	14.3	1.0	27.1	[10]
	42.6	26.4	0.5	1.8	0.6	18.9	[9]
Birch wood	41.5	15.0	1.8	3.0	2.1	25.2	[9]
Douglas fir wood	46.1	3.9	1.1	14.0	2.7	27.3	[11]

*Total *hemicellulose* amount present in lignocellulosics on the basis of % of dry material- Sugarcane bagasse, 27.5; Switch grass, 30; Corn stover, 26.8; Wheat straw, 50; Pine, 26; Aspen, 29; Spruce, 26; Birch wood, 23; Salix wood, 21.7; Douglas fir wood, 20.3.

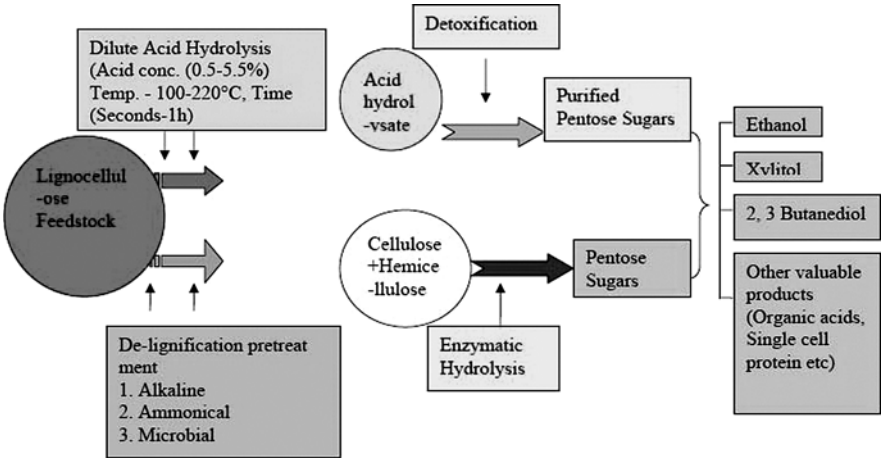

Fig. 1 Mechanistic steps involved in hemicellulose bioconversion into ethanol, xylitol and 2, 3-butanediol

industry. However, several yeast species have the basic ability to carry out these processes, i.e., *Candida shehatae, Pichia stipitis,* and *Pachysolen tannophilus* for ethanol production; *C. utilis*, *C. intermedia*, and *C. gulliermondii* for xylitol production; and *Klebsiella oxytoca* ATCC 8724, *Bacillus subtilis* (Ford strain), and *Aeromonas hydrophilia* for 2, 3-butanediol production [4]. This chapter presents significant advancements in hemicellulose biotechnology, with an emphasis on acidic and enzymatic hydrolysis and the conversion of hemicellulose hydrolysates into commercial products like ethanol, xylitol, and 2, 3-BD.

2 Background Research

To reduce the production of greenhouse gases and ensure sustainable global economic development, it is important to increase the use of renewable biomass resources [14]. There have been active movements accelerating the utilization of lignocellulose-derived products such as bioethanol, xylitol, microbial enzymes, and 2, 3-BD into alternative source of bioenergy [4, 15, 16]. Ethanol has drawn the most attention due to its rapid consumption and the global price fluctuations of crude petroleum [15, 17].

Due to developments in industrial biotechnology, the carbohydrate fraction of the cell wall can be converted into products of industrial significance. However, hemicellulose has been explored less extensively than cellulose due to several factors. The hemicelluloses in lignocellulosic materials are broken down into fermentable sugars by either chemical or enzymatic hydrolysis [18]. The latter is a promising method that breaks down hemicellulosic materials into fermentable sugars without

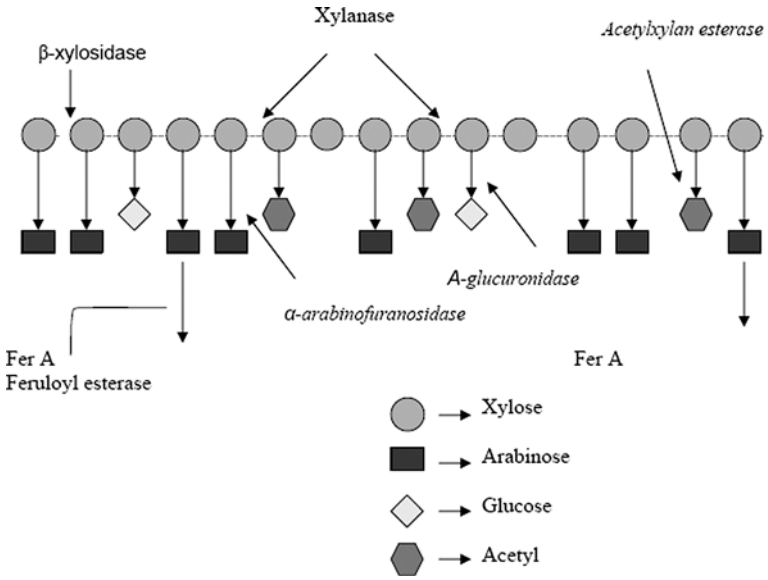

Fig. 2 Schematic presentation of coordinate action of hemicellulases on hemicellulose backbone into monomeric components

increasing the concentration of any inhibiting compounds in the hydrolysate, summarized in Fig. 2. These compounds are produced from hemicellulose hydrolysates by specialized microorganisms under a battery of cultivation techniques.

3 Technical Details – Materials and Methods

3.1 Hemicellulose Hydrolysis

In contrast to cellulose, which is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It is easily hydrolyzed by dilute acid or enzymatically using an arsenal of hemicellulase enzymes [19]. In addition, the lignocellulose can be mildly pretreated with chemicals prior to enzymatic hydrolysis for better saccharification into fermentable sugars. This reduces the crystallinity of the biomass and makes it more amenable to further coordinated enzymatic reactions [18, 20]. Various pretreatment strategies with dilute acid, alkali, ammonia fiber explosion, hydrogen peroxide, steam explosion, wet oxidation, liquid hot water, sodium sulfite, etc., have been discussed [3, 21].

3.1.1 Dilute Acidic Hydrolysis

Dilute sulfuric acid hydrolysis is a favorable method for pretreatment before enzymatic hydrolysis and also for the conversion of lignocellulose to sugars [22].

Compared to other pretreatment methods, it is especially useful for the conversion of hemicellulose into xylose, which can be fermented into ethanol by specialized microorganisms [3, 4]. Most dilute acid processes are limited to a sugar recovery efficiency of around 50%. It has been reported that the cell wall structure and components may be significantly different in different plants, which may influence the digestibility of the biomass [23]. A broad dilute acidic hydrolysis on a variety of lignocellulosic materials with respective ethanol production has been reviewed by Chandel et al. [3].

Formation of Inhibitors During Acid Hydrolysis

During acid hydrolysis of lignocellulosics, aliphatic acids (acetic, formic, and levulinic acid), furan derivatives, and phenolic compounds are formed in addition to the sugars. Furfural and 5-hydroxymethyl furfural (HMF) are the most important furans, formed by decomposition of pentoses and hexoses respectively [24]. Acetic acid has been reported in the hydrolysis of the acetyl groups into hemicellulose as a consequence of deacetylation of acetylated pentosan [25]. Multiple phenolic compounds are derived from lignin, including vanillin, vanillic acid, vanillyl alcohol, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, coumaric acid, syringaldehyde, syringic acid, cinnamaldehyde, dihydroconiferyl alcohol, hydroquinone, catechol, veratrole, acetoguaiacetone, homovanillic acid, and Hibbert's ketones [25]. HMF is converted at a lower rate than furfural, which may be due to lower membrane permeability and cause a longer lag-phase in the growth of microorganisms [26]. The phenolic compounds penetrate biological membranes and cause them to lose integrity, thereby affecting the membranes' ability to serve as selective barriers. The microbial growth was found to be inhibited in the presence of acetic acid (>3.5 g/l) in hemicellulosic hydrolysates, this phenomenon may occur due to the inflow of undissociated acid into cytosol [26].

Removal of Fermentation Inhibitors from the Hemicellulosic Hydrolysates

In order to enhance the efficiency of hydrolysate fermentation, several detoxification methods have been employed, including chemical, physical, and biological methods [25]. These methods include neutralization, overliming, use of ion exchange resins, adsorption onto activated charcoal or tin oxides, and treatments with enzymes such as peroxidase and laccase [3, 25]. Since detoxification increases the cost of the process, it is important to either overcome the need for detoxification steps or develop cheap and efficient detoxification methods. Overliming with CaO or $Ca(OH)_2$ is a classical chemical detoxification method. It efficiently removes furans and phenolics with marginal loss of sugars [24]. Organic solvents such as ether or ethyl acetate have also been applied to extract most of the inhibitors, such as phenolics, weak acids, and furans [25].

Activated charcoal treatment is an efficient and economical method of removing phenolic compounds, acetic acid, aromatic compounds, furfural, and HMF by adsorption [25]. Biological detoxification is another method that enhances the

fermentability of hydrolysates, substantially eliminating phenolic compounds. An enzymatic method using laccase was developed to eliminate the impurities of phenolic monomers and phenolic acids from hemicellulosic hydrolysates of sugarcane bagasse [24].

3.1.2 Enzymatic Hydrolysis

Hemicellulases, which catalyze the hydrolysis of plant cell polysaccharides, are multi-domain proteins generally containing structurally discrete catalytic and non-catalytic modules [27]. The most important non-catalytic modules consist of carbohydrate binding domains (CBD), which facilitate the targeting of the enzyme to the polysaccharide, interdomain linkers, and dockerin modules. The dockerin modules mediate the binding of the catalytic domain via cohesion-dockerin interactions, either to the microbial cell surface or to enzymatic complexes such as the cellulosome [27, 28].

The coordinated action of hemicellulases is necessary to obtain a satisfactory yield of pentose sugars from lignocellulosic as summarized in Fig. 2. Therefore, the development of low-cost and commercial hemicellulases is expected to be a limelight research area for cellulosic ethanol production. Table 2 shows the hemicellulase titers from different microorganisms and their mechanistic applications [29].

3.2 Hemicellulose Hydrolysates into Products of Industrial Significance

3.2.1 Ethanol

Bioethanol is a clean-burning (emits less CO_2 and other green house gasses due to availability of free O_2), non-petroleum liquid fuel that is considered to be a safe supplement to gasoline for transportation. The production and combustion of ethanol do not contribute to the total amount of carbon dioxide in the atmosphere [3, 21]. Ethanol can be mixed with gasoline in 10% (E10), 20% (E20), and 22% (E22) blends without engine modifications, but higher-level blends (such as 85% or 95%) require some engine modification. As a fuel additive, ethanol provides oxygen to the fuel, thus improving fuel combustion and reducing tailpipe emissions of carbon dioxide and unburned hydrocarbons.

Microorganisms

One of the main industrial uses of microorganisms has been alcoholic fermentation. The giant "microbial libraries" in current vogue can be studied for microbes that convert cheaper carbohydrates into value-added products, which can serve as raw materials for the fermentation of hemicellulosic-derived sugars into valuable commercial commodities [30]. The bioconversion process holds more promise of utilizing both hexose and pentose sugars from lignocellulosic materials. Microbial

Table 2 Hemicellulase titers from different microorganisms and their mechanistic applications (*Source*: Howard et al. [29].)

Microorganism	Enzyme	Substrate	Specific activity (μmol min^{-1} mg^{-1})	Mechanistic applications
Bacteria				
Fibrobacter succinogenes	Acetyl xylan esterase	Acetylxylan/alpha-naphthyl acetate	2,933	Hydrolyze the acetyl substitutions on xylose moieties
Thermoanaerobacter ethanolicus	Beta-1,4-xylosidase	o-nitrophenyl-beta-D-xylopyranoside	1,073	Hydrolyse xylobiose; release xylose
Bacillus polymyxa	Beta-Glucosidase	4-nitrophenyl-beta-D-glucopyranoside	2,417	Act upon Beta-Glucosidase to release glucose
Bacillus subtilis	Endo-alpha-1,5-arabinanase	1,5-alpha-L-arabinan	429	hydrolase activity, hydrolyzing O-glycosyl compounds
Escherichia coli	alpha-Galactosidase	Raffinose	27,350	Hydrolyzes the terminal alpha-galactosyl moieties from xylans
Clostridium stercorarium	Feruloyl esterase	Ethyl ferulate	88	Hydrolyze the ester bond between the arabinose substitutions and ferulic acid
Bacillus subtilis	Endo-galactanase	Arabinogalactan	1,790	Release of L-arabinose substituted D-galactooligosaccharides from arabinogalactan
Bacillus subtilis	Endo-beta-1,4-mannanase	Galactoglucomannan/ glucomannans/mannan	514	Acts upon interior side of beta-1,4-mannan to yield mannose
Fungi				
Phanerochaete chrysosporium	Alpha-Glucuronidase	4-O-methyl-glucuronosyl-xylotriose	4.5	Hydrolyses Alpha-1,2 Glycosidic bond the 4-O-methyl-D-glucuronic acid sidechain of xylans

Table 2 (continued)

Microorganism	Enzyme	Substrate	Specific activity (μmol min^{-1} mg^{-1})	Mechanistic applications
Aspergillus niger	Alpha-L-arabinofuranosidase	alkyl-alpha-arabinofuranoside/ aryl-alpha-arabinofuranoside/ Larabinogalactan/ L-arabinoxylan/ methylumbelliferyl-alpha-L-arabinofuranoside	396.6	Hydrolyzes arabinoxylan from terminal alpha-arabinofuranose
Aspergillus niger	Exo-beta-1,4-mannosidase	p-nitrophenyl-beta-D-galactoside	188	Acts upon outer side of beta-1,4-mannan
Trichoderma longibrachiatum	Endo-1,4-beta-xylanase	Beta-1,4-D-xylan	6,630	Cleaves interior Beta-1,4 linkage of xylan backbone

conversion of hexose sugars into chemicals is well established; however, the ability of these organisms to ferment pentose sugars is somewhat less so. The useful exploitation of lignocellulosics by fermentation can be enhanced by efficient utilization of the pentosanic fraction along with hexoses.

Yeasts that have been studied extensively for use in xylose fermentation include *Pachysolen tannophilus*, *Candida shehatae*, *Pichia stiptis,* and *Kluveromyces marxianus* [3]. The optimal performance of these microorganisms is usually controlled by the air supply. Other yeasts investigated for their xylose-fermenting ability include *Brettanomyces*, *Clavispora*, *Schizosaccharomyces*, several other species of *Candida* viz. *C. tenius*, *C. tropicalis*, *C. utilis*, *C. blankii*, *C. friedrichii*, *C. solani,* and *C. parapsilosis*, and species of *Debaromyces* viz. *D. nepalensis* and *D. polymorpha*. Maleszka and Schneider [31] screened 15 yeast strains for their ability to utilize D-xylose, D-xylulose, and xylitol for ethanol production under aerobic, microaerobic (low aeration), and anaerobic conditions using rich undefined or defined media. In almost all cases, ethanol production by *P. tannophilus* and species belonging to *Candida* and *Pichia* was better on rich media under microaerobic conditions [3, 4, 31].

Several pentose-utilizing fungal species like *Fusarium oxysporum*, *Rhizopus* sp., *Monilia* sp., *Neurospora crassa*, *Paecilomyces* sp., *Mucor* sp., *Neurospora crassa,* and *F. oxysporum* and bacterial species like *Bacillus macerans*, *B. polymyxa*, *Kiebsiella pneumoniae*, *Clostridium acetobutylicum*, *Aeromonas hydrophila*, *Aerobacter* sp., *Erwinia* sp., *Leuconostoc* sp., *Lactobacillus* sp., *Clostridium thermocellum*, *C. thermohydrsulfurium*, *C. thermosaccharolyticum*, and *C. thermosulfurogenes* utilizing pentose, hexose, and lignocellulose hydrolysates for ethanol production have been extensively reviewed [32].

Fermentation Methodologies

Researchers have performed all three fermentation processes (batch, fed-batch, and continuous) for biomass conversion into ethanol. The most suitable fermentation strategy depends upon the growth kinetics of the microorganism, the type of hydrolysate, and the economics of the process. For ethanol production from lignocellulosic biomass, batch fermentation has been extensively utilized in the past. The batch process is a multivessel approach that allows flexible operation and easy control in the bioconversion process [33]. In fed-batch fermentation, the microbial cells can be acclimatized at low substrate concentrations that later assist in accelerating the rate of ethanol formation during the entire course of the bioconversion process. Fed-batch fermentation processes are ideal to obtain a high cell density, which may help to achieve higher ethanol yields with greater productivity. Higher cell density also helps to reduce the toxicity of lignocellulose hydrolysates, particularly acid hydrolysates, to yeast cells. Continuous fermentation is another state-of-the-art technology in which microorganisms work at a lower substrate concentration, maintaining higher ethanol concentration during the entire course of the fermentation reaction [34]. Table 3 summarizes the fermentation profiles of different microorganisms utilizing a variety of lignocellulose hydrolysates.

Table 3 Fermentation of various hemicellulosic hydrolysates for ethanol production by different microorganisms

Lignocellulose material	Hydrolysis conditions	Released sugars (g/L)	Detoxification	Microbial strains	Ethanol yield (g/g)	References
Sugarcane bagasse	(2.5% (v/v) HCl, 140°C, 30 min), # Solid:liquid = 1:10	30.29	Overliming + ion exchanger	*C. shehatae* NCIM3501	0.48	[24]
Rice straw	Presoaking with 0.5% of H_2SO_4 for 18 h followed by steam heating at 15 bar pressure for 10 min, Solid:liquid = 600 g:4 L of 0.5% H_2SO_4	228 g sugar/Kg of substrate	Overliming	*Mucor indicus*	0.24	[35]
Wheat straw	(90°C, 1.85% (w/v) H_2SO_4, 18 h), Solid:liquid= 1:20	17.10	Overliming	*P. stipitis* NRRL Y-7124	0.41±0.01	[36]
Corn cob	(0.3 M H_2SO_4, 98°C, 1 h), Solid:liquid = 1:12	45.0	Overliming +ZSM-39 shaking	*P. stipitis*	0.44	[37]
Eicchornia crassipies	(1% (v/v) H_2SO_4, 7 h), Solid:liquid = 1:8	67.5	Overliming + sodium sulfite	*P. stipitis* NRRL Y-7124	0.35	[38]
Pine	Sulfur dioxide (30 min, 160°C + (225°C, 30 s, HCI equivalent to 1% of dry weight), Solid:liquid = 1:3	72	Overliming + sodium sulfite	*E. coli* K011	0.43	[39]

Table 3 (continued)

Lignocellulose material	Hydrolysis conditions	Released sugars (g/L)	Detoxification	Microbial strains	Ethanol yield (g/g)	References
Willow	Steam (1 bar pressure, soaked with gaseous SO, (1 g SO_2/100 g willow) 6 min, 206°C), Solid:gas = 100:1 g	9.0	Overliming + sodium sulfite	*E. coli* K011	0.51	[40]
Mixed wood	# # Acid hydrolysis	70.4	Electrodialysis + Sodium hydroxide	*C. shehatae* FPL-Y-049	0.48	[41]
Paja brava	Pre-steamed, impregnated with dilute sulfuric acid (0.5% or 1.0% by wt), + hydrolysis at temperatures between 170 and 230°C for a reaction time between 3 and 10 min. Solid:liquid = 1:10	22.2	No Detoxification	*P. stipitis* CBS 6054	0.20	[42]

Solid:liquid (Lignocellulose substrate: dilute acid solution).
Details are not available.

3.2.2 Xylitol

Xylitol is a naturally-occurring sugar with a wide spectrum of potential applications. It has a sweetening power matching that of sucrose (table sugar), and is used as a sugar substitute in the food processing industry [43]. Xylitol produces a perceived sensation of coolness in the mouth as it comes in contact with saliva because of its negative heat of solution [43]. Xylitol can be produced through microbial transformation reactions by yeast from D-xylose, or by both yeast and bacteria from D-glucose [44]; D-xylose can also be directly converted into xylitol by NADPH-dependent xylose reductase [45].

Microorganisms

Xylitol can be produced by bacteria and filamentous fungi [46], but often the best producers are yeasts, especially species of the genus *Candida,* such as *C. guilliermondii, C. pelliculosu, C. parapsilosis,* and *C. tropicalis* [47, 48]. Other yeast genera investigated for xylitol production from xylose include *Saccharomyces, Debaryomyces, Pichia, Hansenula, Torulopsis, Kloeckera, Trichosporon, Cryptococcus, Rhodotorula, Monilia, Kluyveromyces, Pachysolen, Ambrosiozyma,* and *Torula* [45]. Bacteria species such as *Enterobacter liqufaciens*, *Corynebacterium* sp., and *Mycobacterium smegmatis* [46] can also produce xylitol. The conversion of D-xylose to xylitol by microorganisms is important for industrial production and has been studied extensively in yeasts, as summarized in Table 4.

Fermentation Methodologies

Batch fermentation has been explored extensively for the production of xylitol *(47)*. Laboratory-based investigations in culture flasks did not show significant xylitol production. A higher substrate concentration is mandatory to obtain the genuine yield of xylitol in batch fermentation. Further studies will help to define the mechanism of xylitol fermentation under the desired set of fermentation reactions. The higher level of end products like ethanol, biomass and carbon dioxide in the media may also inhibit xylitol production [47].

In fed-batch operations, a constant substrate concentration can be maintained during the course of fermentation [48]. *C. boidinii* NRRL Y-17231 fermentations showed 75% theoretical xylitol yield in a fed-batch process, compared to 53% theoretical yield in a batch process [47]. Alternatively, continuous culture techniques have shown higher productivity with increased xylitol yields from several microorganisms. Feeding of nutrient media with an optimized dilution rate is a critical parameter in continuous cultures that helps achieve the higher rate of xylitol production. Table 4 lists a variety of microbial strains producing xylitol using different lignocellulosic sources.

Table 4 Fermentation of various hemicellulosic hydrolysates for xylitol production by different microorganisms

Lignocellulose material	Hydrolysis conditions	Sugars in hydrolysate (g/L)	Detoxification strategy	Microbial Strain	Xylitol Yield (g/g)	References
Sugarcane bagasse	1% (v/v) H_2SO_4, 120°C, 1 h, Solid:liquid = 1:5	30.0	Activated charcoal + Ion exchanger	*C. tropicalis*	0.65	[47]
Rice straw	126°C, 1% (v/v) H_2SO_4, 90 min, Solid:liquid = 1:10	20.7	Calcium hydroxide + Activated charcoal	*C. subtropicalis* WF79	0.73 g	[49]
Wheat straw	140°C, 30 min, Solid:liquid = 0.2:1	0.26 g sugar/g of wheat straw	Activated charcoal	*C. guilliermondii* FTI 20037	0.90	[50]
Brewer's spent grain	1.25% (w/v) H_2SO_4, 120°C, 17 min, Solid:liquid = 1:8	70	Alkali treatment	*C. guilliermondii*	0.78	[51]
Brewer's spent grain	2% (w/w) H_2SO_4, 121°C for 15 min, Solid:liquid = 1:8	26.3	Activated charcoal	*Debaryomyces hansenii* CCMI 941	0.50	[52]
Eucalyptus grandis	0·5% H_2SO_4, 140°C for 10 min, Total immersion time in acid solution 24 h and treated with CaO	54.7	Calcium hydroxide + NaOH	*C. guilliermondii* FTI 20037	0.54	[53]
Corn fiber	1% (v/v) H_2SO_4, 120°C, 1 h, Solid:liquid = 1:5	30.0	Activated charcoal + ion exchanger	*C. tropicalis*	0.58	[47]
Mixed wood	3.5% H_2SO_4, normal boiling temperature, 11 h	58–78	Activated charcoal	*D. hansenii* NRRL Y-7426	0.73	[54]

3.3 2, 3-Butanediol

2, 3-BD is the 2R, 3R isomer of 1, 4-butanediol, a potential bulk chemical that can be produced by a variety of microorganisms through microbial fermentation [55]. It has been utilized for the production of various chemical feedstocks and liquid fuels, including the formation of the liquid fuel additive methyl ethyl ketone by dehydration [56]. The esters of butanediol and suitable monobasic acids may find uses as effective plasticizers for thermoplastic polymers, such as cellulose nitrate and cellulose triacetates [55].

3.3.1 Microorganisms

Fermentation of xylose and glucose by *Klebsiella oxytoca* and *Aerobacter aerogenes* yields 2, 3-BD as the major product [55]. Other microorganisms capable of producing 2, 3-BD include *Bacillus subtilis* (Ford strain), *Aeromonas hydrophilia,* and several *Serratia* sp. [55]. *K. oxytoca* is able to yield high concentrations of 2, 3-BD as mixtures of stereoisomers from monosaccharides, but is unable to utilize polysaccharides. In comparison, *B. polymyxa* is able to ferment starch directly, yielding 2, 3-butanediol and ethanol in almost equal amounts [55].

3.3.2 Fermentation Methodologies

The efficiency of 2, 3-BD fermentation can be judged by the product yield from sugar, the final butanediol concentration, and the volumetric butanediol production rate. The theoretical yield of 2, 3-BD from glucose is 0.50 g/g. Higher levels of butanediol have been produced in fed-batch culture conditions that are maintained to minimize the effects of initial substrate inhibition and product inhibition. A higher production rate of 2, 3-BD was reported in continuous reactors [55]. However, product inhibition and incomplete substrate utilization remain challenging issues. Immobilization of live cells on a supporting material, i.e., matrix, has been attempted to increase the total yield of 2, 3-BD. In terms of overall performance, a two-stage continuous immobilized live cell reactor was found to be the most efficient for 2, 3-BD formation [55, 57].

The single greatest cost in most biomass conversion processes is the substrate cost [1, 2]. Hence, an inexpensive carbohydrate substrate is essential to develop an economical fermentation process for the production of 2, 3-BD. Different carbohydrate sources used by microorganisms producing 2, 3-BD under different culture conditions were reviewed [55]. pH is a crucial parameter during 2, 3-BD formation. A pH range from 5 to 6 was found to be optimal for accelerating the formation of 2, 3-BD by *K. oxytoca* [58]. In addition, a microbial growth temperature (i.e. 37°C) at which the sugar uptake can be managed by increasing the rate of 2, 3-BD formation is absolutely necessary [55]. Another important variable that affects the yield of 2, 3-BD and the productivity of the microorganisms is the rate of oxygen flow in the fermentation reaction [55]. These factors significantly contribute to 2, 3-BD

production, and they present the most challenges to maintaining a constant rate of 2, 3-BD formation during the entire course of the fermentation reaction.

3.4 Other Products

Besides ethanol, xylitol, and 2, 3-BD, other industrially significant products such as lactic acid, itaconic acid, and single cell protein (SCP) can be manufactured using hemicellulose sugars. These products have wide applications in the food, feed, pharmaceutical, and cosmetics industries. Garde et al. [59]. reported lactic acid production from wet-oxidized wheat straw by *Lactobacillus brevis* and *L. pentosus.* Sugar cane bagasse hemicellulosic hydrolysate was converted into lactic acid by thermotolerant acidophilic *Bacillus* sp. in a simultaneous saccharification and fermentation approach [60].

SCP production from hemicellulose is another cutting-edge area in hemicellulose biotechnology. Microorganism *Candida blankii* UOVS-64.2 was employed for SCP production from hemicellulose hydrolysates, and was increased by intraspecific protoplast fusion of auxotrophic mutants produced by UV irradiation followed by nystatin enrichment [61]. Pessoa et al. [62] showed microbial protein production from sugar cane bagasse hemicellulosic hydrolysate using *Candida tropicalis* IZ 1824 with a net cell mass of 11.8 g L^{-1} and a yield coefficient ($Y_{x/s}$) of 0.50 g g^{-1}.

4 Expert Commentary and Five-Year View

The current shortages and high prices of gasoline products are making it clear that a sustainable, economical, and environmentally benign process for producing fuel is needed. In the future, lignocellulosic-derived products are poised for sharp growth. According to a recent McKinsey report, the bio-based products market is expected to exceed $182.91 billion by 2015 [34]. Lignocellulosic-derived products may play a pivotal role to match this expectation and future markets seem very promising for ethanol, xylitol, organic acids, and 2, 3-BD. Mechanisms for higher yield and productivity of these value-added products can be developed by exploring the hemicellulose fraction of the cell wall in depth.

The fermentation of pentose sugars is not as easy as that of cellulosic-derived hexose sugars due to the unavailability of appropriate microorganisms and the lack of an established bioconversion process. In-depth studies of methods for hemicellulosic degradation are required. This will assist in limiting the role of fermentation inhibitors during hemicellulosic degradation. In the past five years, there has been substantial development in the area of hemicellulose hydrolysis using routine methodologies with known microorganisms. A newer approach to hydrolyzing technologies using a battery of hemicellulase titers needs to be developed to produce high yields of sugar monomers and eventually convert them into value-added products. Isolation and screening of potent hemicellulase-producing microorganisms and further development of mutants/cloned microorganisms may improve the

production yields of the desired titers on a commercial scale. Genetic engineering may also improve microbial efficiency for the overproduction of industrial products using cheaper sources of carbohydrates in fermentation media, the hallmark of commercial fermentation processes. The microbes will be more useful if they have characteristics such as thermotolerance, alkalotolerance, or tolerance of other extreme conditions.

Hemicellulose degradation into fermentable sugars is another area where the scope of research seems enormous. Efforts are underway at our laboratory for the production of ethanol and xylitol from lignocellulose feedstock. Multiple research projects are being sponsored by government agencies to improve the pretreatment process of lignocellulosics for their conversion into ethanol and xylitol [24, 63–69].

In the last five years, there has been comparatively less research into 2, 3-BD production than into ethanol and xylitol production worldwide. New research insights, such as the development of transgenic plants containing less lignin, may be helpful for the conversion of biomass into value-added products. Chen and Dixon [70] developed antisense-mediated down-regulation of lignin biosynthesis in alfalfa to reduce or eliminate the need for pretreatment. This may make the hemicellulosic fraction more accessible due to the reduced presence of lignin, which in turn will require a milder pretreatment and less enzymatic load to get the desired yield of fermentable sugars. Releasing genetically engineered plants may raise ethical issues among environmentalists; however, it can be assumed that the generation of new products from hemicellulose will strengthen the economy by saving foreign exchange reserves and promoting energy independence, which will benefit the environment.

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